Environmental Health Perspectives
Vol. 65, pp. 255-262, 1986

Genetic Toxicology of Phthalate Esters: Mutagenic and Other Genotoxic Effects

by George R. Douglas,* Alida P. Hugenholtz,*† and David H. Blakey*

The effects of DEHP on sperm morphology and on peripheral blood micronuclei were studied for 12 weeks following five subacute IP injections of DEHP at 1/6, 1/12, and 1/60 of the LD_{50} per day. Sperm morphology was examined in both adult mice and rats, while peripheral blood micronuclei were scored in mice up to 4 weeks after treatment. In mice, DEHP at 1/6 LD_{50} significantly depressed body weight gain for up to 12 weeks after treatment, and reduced epididymal sperm number by 4 weeks. Numbers of morphologically abnormal sperm did not differ from controls in the 12 weeks following treatment. In addition, DEHP did not increase the numbers of peripheral blood micronuclei.

Studies in the rat indicated that exposure to doses of 1/6 and 1/12 of the LD_{50} per day of DEHP resulted in a reduced gain in body weight compared to controls. Testis weight, sperm number, and numbers of morphologically abnormal sperm were unaffected by DEHP following treatment. In separate experiments, DEHP did not induce sister chromatid exchange (SCE) or DNA damage in Chinese hamster ovary (CHO) cells. Although DEHP is known to cause testicular atrophy in rats and to a lesser extent in mice, it did not cause an increase in abnormal sperm in either species. Together with the CHO and micronucleus data, these findings suggest that DEHP has a low probability of causing genetic damage capable of being transmitted through the male germ line.

Introduction

A valid decision on the genotoxicity of phthalates, as with any chemical substance, requires data from a carefully chosen battery of tests. Such a battery would ideally contain tests for the two basic types of genetic effects (i.e., gene mutations and chromosomal mutations) with redundancy in terms of cell type or organisms where practicable. In terms of making any decisions concerning potential hazard to human beings, it is imperative to recognize that the results of *in vitro* tests can only demonstrate the potential to cause the critical effects *in vivo*. Thus, the actual confirmation of any *in vitro* effects requires the conduct of the appropriate *in vivo* tests.

Table 1 is a representative, but not necessarily exhaustive, summary of studies which have been reported in the scientific literature on the genotoxicity of phthalates. While it is difficult to generalize about a broad chemical class, certain observations can be made. The *in vitro* and *in vivo* data are equivocal and give no clear-cut indication of genotoxicity. Where redundancy in endpoints with the same chemical does exist, there is often a lack of agreement. Discrepancies in the *in vivo* tests do not seem to be due to differences in the route of administration, since both chromosome aberrations and dominant lethality are observed after topical, subcutaneous, or intraperitoneal injection, as well as following oral administration.

There are extensive data on a number of other indicators of toxicity, many of which are discussed elsewhere in this issue. Examples of some of these effects which relate to the purpose of this paper are shown in Table 2. Apart from the indication of liver tumors and teratogenicity, which may or may not be related to the genotoxic effects observed, there is clear evidence of cytotoxicity both *in vitro* and *in vivo*; the most important perhaps being testicular cell damage (Table 3). Such effects appear to be related to the age and species as well as to the route of administration.

Because the existing data suggested a genotoxic effect and the clear evidence of testicular damage, we reasoned that one of the most appropriate tests for further study of the potential genotoxic effects of phthalic acid esters would be the sperm abnormality assay (*39*). A number of carcinogens and mutagens are known to induce abnormal sperm morphology, and some abnormalities are inherited by the offspring of treated males (*39,40*). However, in addition, increased temperature and food deprivation are also known to induce abnormally shaped sperm (*41,42*). We have conducted a study on both mice and rats using DEHP as a representative phthalate. In order to assist in the determination of the cause of any

*Mutagenesis Section, Environmental Health Directorate, Department of National Health and Welfare, Environmental Health Centre, Ottawa, Canada, K1A OL2.

†Present address: Toxicological Evaluation Division, Foods Directorate, Department of National Health and Welfare, Ottawa, Canada.

Table 1. Survey of reported genotoxic effects of phthalate esters.

Test	Phthalate	Organism	Result	Reference
Gene mutation	DEHP	*Salmonella typhimurium* strains TA 1535, 100, 1537, 1538, 98	− (±S9)	Simon et al. (*1*)
	DHP		− (±S9)	
	DIBP		− (±S9)	
	MEHP	*S. typhimurium* strains TA 1535, 100, 1537, 1538, 98	− (±S9)	Ruddick et al. (*2*)
	DEHP	*S. typhimurium* strains TA 98, 100 (modified)	− (±S9)	Kozumbo et al. (*3*)
	MEHP		− (±S9)	
	DBP		− (±S9)	
	BBP		− (±S9)	
	DMIP		− (±S9)	
	DMTP		− (±S9)	
	DMP *ortho* esters		+ (−S9)	
	DEP		+ (−S9)	
	20 phthalates and related chemicals	*S. typhimurium* strains TA 1535, 100, 1537, 1538, 98 (preincubation)	− (±S9)	Zeiger et al. (*4*)
	MEHP	*S. typhimurium* strain TA 100	+ (−S9)	Tomita et al. (*5*)
		E. coli strain WPI	+ (−S9)	
	DEHP	*S. typhimurium* strain TA100	+ (+S9)	
	DMP	*S. typhimurium* strain TA 100 (liquid suspension assay)	+ (±S9)	Seed (*6*)
	DEP		+ (±S9)	
	DAP		− (±S9)	
	DBP		+ (±S9)	
	DOP		− (±S9)	
	DEHP		− (±S9)	
	DEHA		− (±S9)	
	2-ethylhexanol		+ (±S9)	
	DIDP		− (±S9)	
	DIBP		− (±S9)	
	MEHP	CHO cells $HGPRT^-$ locus	inconsistent +/− (−S9)	Phillips et al. (*7*)
	DEHP	Mouse lymphoma L5178Y $TK^{+/-}$ locus	− (±S9)	Kirby et al. (*8*)
	DEHP (oral) MEHP	Syrian golden hamster embryonic cells (*in vivo/in vitro*)	+	Tomita et al. (*5*)
Chromosome aberrations	DEHP	Chinese hamster Don cells	− (−S9)	Abe and Sasaki (*9*)
	DBP		− (−S9)	
	DEHP	CHO cells	− (−S9)	Phillips et al. (*7*)
	MEHP		+ (−S9)	
	2-ethylhexanol		+/− (−S9)	
	DEHP	Human leukocytes in culture	− (−S9)	Tsuchiya and Hat tori (*10*)
	DBP		−	
	DEP		−	
	DMP		−	
	DEHP	Human fetal lung in culture	− (−S9)	Stenchever et al. (*11*)
	DMP (topical)	Rat hepatocytes	+ (multiple doses) − (single dose)	Yurchenko (*12*)
	MEHP	Syrian hamster V79 cells	+ (+S9)	Tomita et al. (*5*)
		Syrian golden hamster embryonic cells (*in vivo/in vitro*)	+	
SCE	DEHP	Chinese hamster Don cells	+ (not dose	Abe and Sasaki (*9*)
	DBP		+ related)	
	MEHP	CHO cells	−	Phillips et al. (*7*)
Dominant lethality	DEHP (IP)	ICR mouse	+ (over 12 wks)	Singh et al. (*13*)
	DMEP (IP)		+ (1st 3 wks)	
	DEHP (oral)	Mouse	− (over 6 wks)	Hamano et al. (*14*)
	MEHP (oral)		− (over 6 wks)	
	DEHP (sc)	Mouse	+ (preimplant.)	Autian (*15*)

[a] Abbreviations for Tables: DEHP, di-(2-ethylhexyl) phthalate; DHP, dihexyl phthalate; DIBP, diisobutyl phthalate; DIDP, diisodecyl phthalate; MEHP, mono-(2-ethylhexyl) phthalate; DBP, dibutyl phthalate; BBP, butyl benzyl phthalate; DMIP, dimethyl isophthalate; DMTP, dimethyl terephthalate; DMP, dimethyl phthalate; DEP, diethyl phthalate; PA, phthalic acid; DAP, diallyl phthalate; DEHA, di (2-ethylhexyl) adipate; DPeP, di-*n*-pentyl phthalate; DPP, dipropyl phthalate; DMEP, dimethoxyethyl phthalate.

Table 2. Some reported cytotoxic, teratogenic and carcinogenic effects of phthalate esters.

Test	Phthalate	Organism	Result	Reference
Cytotoxicity	9 phthalates	Rat cerebellum fibroblasts	+/− (high mw, low H_2O solubility is most toxic)	Teranishi and Kazuya (*16*)
	DMEP	Replicating mouse L cells	+	Dillingham and Autian (*17*)
	DEHP	L cells	−	Gesler (*18*)
	DMP		+	
	Various phthalates		?	
	DEHP	CHO cells	−	Phillips et al. (*7*)
	MEHP		+	
	2-Ethylhexanol		+	
	DEHP	Chick embryo cells	+	Lee and Kalmus (*19*)
	DEHP	Chick embryo	−	Gesler (*18*)
	DMP		+	
	Various phthalates		+/−	
	DEHP	Aquatic invertebrates	+/− (low toxicity, high	Saunders et al. (*20*)
	DBP		+/− excretion rate)	
Embryotoxicity	DEHP (diet)	ICR mouse	+	Shiota et al. (*21*)
	DBP		+ (low birth weight)	
Teratogenicity	DEHP (diet)	ICR mouse	+ (skeletal neural tube	Shiota et al. (*21*)
	DBP		+ malform.)	
	DEHP (oral)	Mouse d7–8	+ (gross	Yagi et al. (*22*)
	MEHP		+ and skeletal)	
	DEHP (IP)	Rat	− (skeletal)	Singh et al. (*23*)
	DMP (IP)		+ (skeletal)	
	DMEP (IP)		+ (skeletal)	
	MEHP (gavage)	Rat d6–15	+ (fetotoxicity)	Ruddick et al. (*2*)
	Phthalates (yolk sac)	Chick embryo	various effects	Haberman et al. (*24*)
Carcinogenicity	DEHP (diet)	F344 rat	+ (hepatocellular	NTP (*25*)
		$B_6C_3F_1$ mouse	+ carcinomas)	
	DMTP (diet)	F344 rat; $B_6C_3F_1$ mouse	Equivocal	NTP (*26*)
	DAP (gavage)	$B_6C_3F_1$ mouse	−	NTP (*27*)
	DEHA (diet)	$B_6C_3F_1$ mouse	+ (hepatocellular carcinomas)	NTP (*28*)
		F344 rat	− (toxic liver effects)	
	Phthalamide	$B_6C_3F_1$ mouse	− (urinary toxicity, no tumors)	NTP (*29*)
	Phthalic anhydride	F344 rat, $B_6C_3F_1$ mouse	−	NTP (*30*)

effects of sperm morphology in the mouse study, circulating red blood cells were assayed for the presence of micronuclei (*43*), the products of a subset of chromosomal damage. In separate *in vitro* experiments with CHO cells, the effect of DEHP on the formation of SCE and the induction of alkaline sucrose gradient-detectable DNA lesions were also determined.

Methods and Materials

Mice of hybrid strain B6C3F1 and Sprague-Dawley strain rats were obtained from Charles River Breeders (Lasalle, Quebec). Mice were 6 to 8 weeks old and weighed 18 to 25 g when treated, while rats of the same age weighed 300 to 375 g when injected. Subacute IP injections of DEHP (Cat. #D20,115-4; Aldrich, Milwaukee, WI) in olive oil (#3016; Eastman Kodak, Rochester, NY; or Fisher Scientific, Fair Lawn, NJ) were administered to each animal, along with untouched and solvent negative controls, and MMS (methyl methanesulfonate; #7936, Eastman Kodak, Rochester, NY), in normal saline as a positive control. The groups consisted of three animals (rats) or five animals (mice) per control group per week and five animals per treatment group per week. Animals were injected on five consecutive days with maximum volumes of 0.2 mL/mouse and 2.5mL/rat. Doses of DEHP administered were as follows: mice, 6.0g/kg/day (⅙LD_{50}),3.0g/kg/day(1/12LD_{50}),0.6g/kg/day(1/60LD_{50});rats; 5.2 g/kg/day (⅙ LD_{50}), 2.6 g/kg/day (1/12 LD_{50}), 0.52 g/kg/day (1/60 LD_{50}). MMS was administered at 75 mg/kg/day for the positive control mice. Since several mice were lost during the course of the study, rats were exposed to 75 mg/kg/day on the first injection day, but to 37.5 mg/kg/day on the remaining four treatment days. The "week 0" group of animals was sacrificed following the injections on the 5th day of treatment and every 2 weeks thereafter for up to 12 weeks. Mice were sacrificed by cervical dislocation and rats by CO_2 asphyxiation.

Body weights and testes weights were recorded at sacrifice, and epididymal sperm smears stained in 1% eosin Y were prepared according to Wyrobek and Bruce (*39*), except that rat epididymes were minced in 10 mL of PBS per cauda. Sperm number was obtained using a hemacytometer. 500 sperm or more were scored for abnormalities per animal. Data are presented as the means for each treatment group, and analyzed using the *G*-test

Table 3. Some reported testicular effects of phthalate esters.

Phthalate	Organism	Result	Parameter	Reference
DEHP	Rat	+	atrophy	Gangolli (31)
DBP				
MBP				
DEHP	Mouse			
DBP				
DEHP	Guinea pig			
DBP				
DEHP	Hamster	−	atrophy	
DBP				
DMP	Rat			
DPP	Rat	+	atrophy	
DHP	Rat		atrophy	
DEHP (IP or SC)	Adult mouse	−	testis weight, Zn	Curto and Thomas (32)
	Adult rat	+	testis weight, Zn	
MEHP (IP or SC)	Adult mouse	−	testis weight, Zn	
	Adult rat	+	testis weight, Zn	
DEHP (diet)	Immature mouse	+	testis weight, Zn	Oishi (30)
DEHP (oral)	4-wk-old rat	+	testis weight, Zn	Gray and Butterworth (34)
	10-wk-old-rat			
	15-wk-old-rat	−		
DEHP (IP)	Immature rat	+	atrophy	Seth et al. (35)
DEHP (diet)	Prepubertal rat	+	atrophy, testis weight	Oishi and Hirage (36)
DBP				
DIBP				
PA				
DBP (oral)	Prepubertal rat	+	atrophy and Zn	Foster et al. (37)
DPeP				
DHP				
DPeP (gavage)	Prepubertal rat	+	Sertoli cell enzymes, atrophy	Creasy et al. (38)

or Students's t-test (44).

In the mouse experiment, 1000 monochromatic erythrocytes/animal were scored for the presence of micronuclei from the 0, 2, and 4 week groups according to the method described by Schlegel and MacGregor (43).

SCE and alkaline sucrose gradient-detectable DNA damage was detected in CHO cells as described previously (45). Cells were treated for 1 hour in minimal MEM minus calf serum with DEHP dissolved in DMSO.

Results

DNA Damage and SCE in CHO Cells

No increase in the level of SCE (Table 4) or in DNA damage detectable by alkaline sucrose gradient analysis (Table 5) was observed after treatment of CHO cells.

Table 4. Effect of DEHP on SCE in CHO cells.

	SCE/cell	
Concentration, mM	+S9	−S9
0	6.72	6.96
0.01	—	7.05
0.05	—	6.75
0.1	6.53	7.17
0.5	7.13	7.03
1.0	7.16	6.80
3.0	7.57	—
6.0	6.73	—
10.0	6.43	—
3.0 DMN	20.8	—
0.5 MMS	—	43.5

Body Weights and Gross Changes

Mice exposed to the highest dose of DEHP (6.0 g/kg/day) showed significant reductions in body weight gain (t-test; $p < 0.05$) compared with other treatment groups and with control groups at the second sacrifice and continuing for the duration of the study ($p < 0.01$) (Fig. 1a). A decline in body weight gain was also seen in rats for 12 weeks after exposure to 5.25 g/kg/day ($p < 0.01$) or 2.6 g/kg/day ($p < 0.05$) (Fig. 1b). Both species showed extensive whitish deposits in the diaphragm, abdominal wall and in the scrotal fat which persisted throughout the study. In the later weeks, the scrotal sac was filled with a milky colored fluid in some of the high and middle dose animals from both species.

Peripheral Blood Micronuclei in Mice

Table 6 presents the results obtained when mice exposed to DEHP were sacrificed 0, 2, or 4 weeks after the final injection. The positive control, MMS, displayed

Table 5. Effect of DEHP on alkaline sucrose gradient-detectable DNA damage in CHO cells.

	Induced breaks/108 daltons	
Concentration, mM	+S9	−S9
10.0	0.019	0
30.0	0	0
50.0	—	0
70.0	0.004	0
100.0	0	0
5.0 DMN	0.965	—
2.0 MMS	—	1.895

a significantly ($p << 0.001$) elevated number of micronuclei on the day of sacrifice following the final treatment (week 0), but levels returned to control values by the second week. No increase in micronuclei was detected at any of the doses or times following DEHP exposure, nor with the solvent or untouched control.

Testis Weight

Mice exposed to 6.0 g/kg DEHP per day showed a decline in testis weight compared to the solvent or untouched controls by 4 weeks after treatment, but the decline was not significant (Fig. 1*a*) except at 8 weeks ($p < 0.05$).

MMS, on the other hand, caused a significant decrease in testis weight 2 weeks ($p < 0.001$) and 4 weeks ($p < 0.01$) after treatment, which then returned to within normal range (Fig. 1*a*). No such decline in testis weight ($p > 0.05$) was seen for any of the treatment groups in the rat as indicated in Figure 1*a*.

Sperm Number

Mice exposed to DEHP at 6.0 g/kg/day showed a significant reduction in sperm number per epididymis from 4 weeks following exposure to the end of the study ($p < 0.01$) (Fig. 1*a*). MMS, on the other hand, showed a reversible decline in sperm number, that attained control values by the final weeks (Fig. 1*a*). By 10 weeks after exposure, sperm numbers in the rats were not significantly reduced ($p > 0.05$) at any of the doses of DEHP (Fig. 1*b*). A decline in sperm number was not significant ($p > 0.05$) following exposure to MMS in the rats.

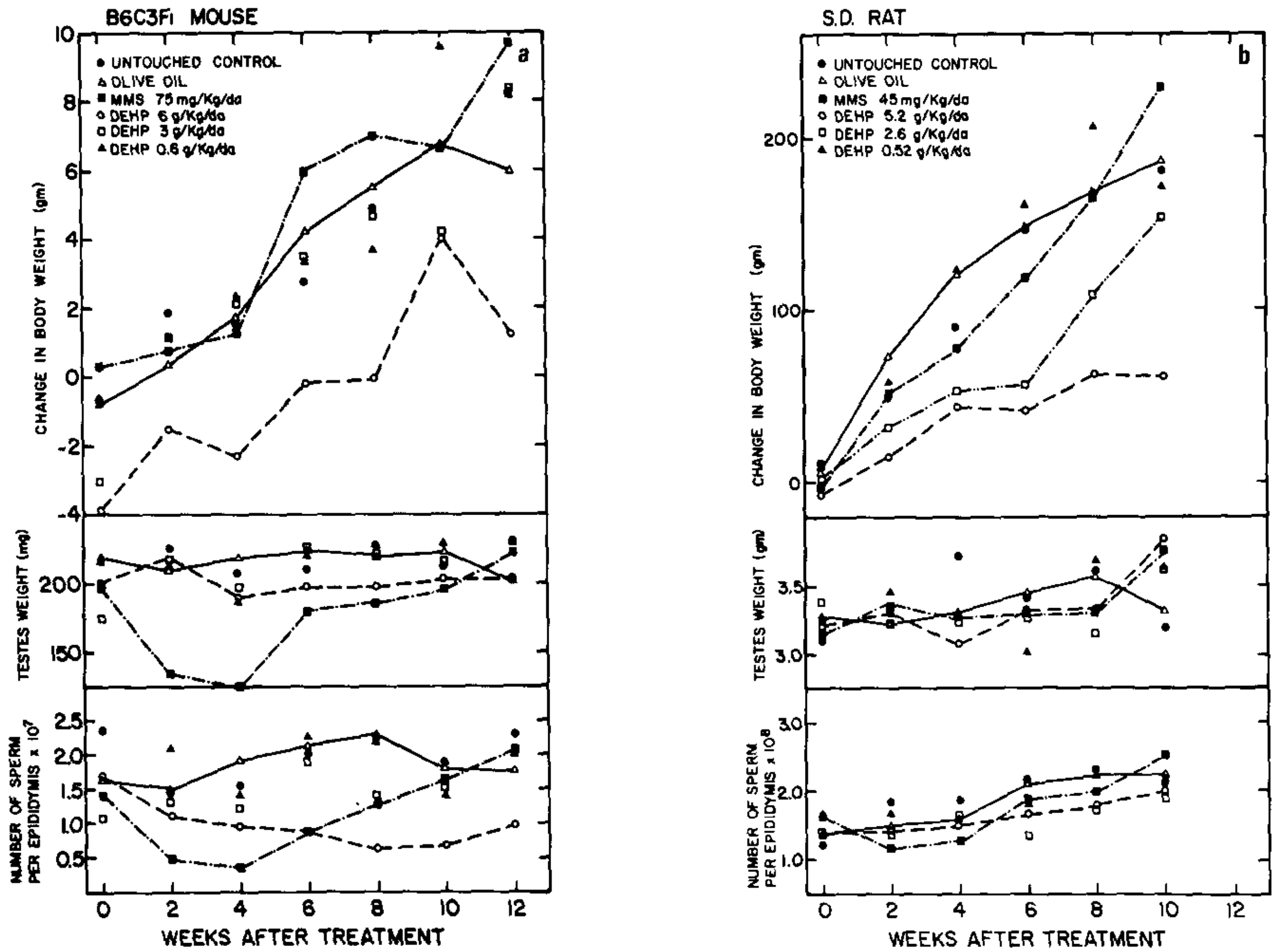

FIGURE 1. Effect of exposure to DEHP on body weight gain, testes weight, and sperm number in mice and rats: each point represents the mean of the treatment groups.

Table 6. Induction of micronuclei in peripheral blood erythrocytes of $B_6C_3F_1$ male mice given five daily injections of DEHP.

Treatment	Micronuclei/1000 erythrocytes at various times following final injection[a,b] 0	2 weeks	4 weeks
Untouched control	4.60 ±1.75 (5)	0.75 ± 0.87 (4)	4.60 ± 1.48 (5)
Solvent control	5.00 ±4.29 (4)	3.00 ± 1.49(4)	1.50 ± 1.00 (4)
0.6 g/kg/day DEHP	3.40 ±1.52 (5)	6.20 ± 1.75 (5)	3.60 ± 0.76 (3)
3.0 g/kg/day DEHP	5.00 ±4.42 (3)	2.80 ± 1.52 (5)	3.17 ± 0.59 (5)
6.0 g/kg/day DEHP	1.20 ±1.34 (5)	2.00 ± 1.22 (5)	2.80 ± 0.96 (6)
75 mg/kg/day MMS	20.75 ±6.85 (4)	2.00 ± 1.22 (3)	2.33 ± 1.47 (3)

[a] Micronuclei/1000 ciruclating normochromatic erythrocytes ± standard error of the mean.
[b] The numbers in parentheses indicate the number of animals analyzed.

Sperm Morphology

The results of the sperm abnormality studies are shown in Figure 2. In general, DEHP did not exert striking increases in the levels of abnormally shaped sperm in either species, over the 12-week period examined.

Discussion

In this investigation, two *in vitro* assays were performed with CHO cells to assist in the determination of genotoxic potential; alkaline sucrose gradient analysis, which detects a spectrum of different types of DNA damage, and the SCE assay, which must be regarded as an indicator of DNA damage. Accordingly, in the interpretation of these data, it must be realized that neither of their effects represent mutations *per se*. Furthermore, as these are *in vitro* tests, their results must be tempered with the wisdom of experimental data from whole animal models. The lack of an effect in the peripheral blood micronucleus assay in mice complements the negative findings of the *in vitro* assays.

The sperm abnormality assay performed both in mice and rats represents a departure from the protocol established by Wyrobek and Bruce (*39,46*), where a single sacrifice time of 5 weeks following the first treatment

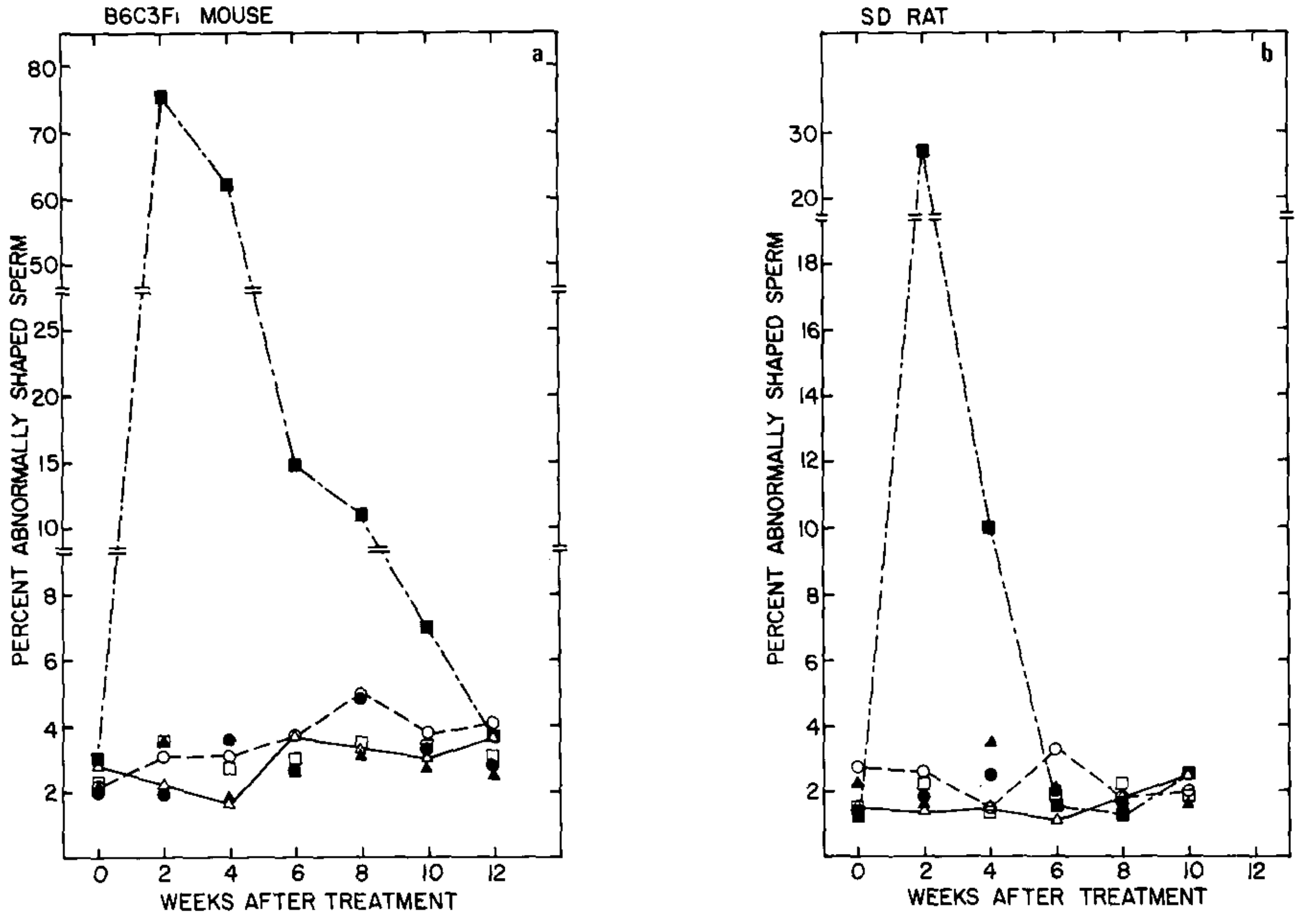

FIGURE 2. Effect of exposure to DEHP on percentage of morphologically abnormal sperm in mice and rats: each point represents the means of the treatment groups.

was employed. In the present study, animals were sacrificed every 2 weeks up to 12 weeks following the last dose. The advantage of this protocol can be seen by the observation that the positive control (MMS) produced the maximum response at 2 weeks in these studies rather than at approximately 4 weeks. These data demonstrate that optimal responses can vary from one agent to another, and point out the necessity of using multiple sacrifice times.

No sperm abnormalities were induced by DEHP in either mice or rats, even though toxic effects were seen in the testis (i.e., reduced testis weight and sperm count in mice) and body weight gains were reduced on both species. To the extent that abnormal sperm morphology is an indicator of genotoxicity (*47*), it would appear that DEHP would have little probability of causing inherited genetic damage in the offspring of treated animals. The other negative data in this study support this conclusion. Furthermore, although toxic effects other than mutagenicity are known to induce sperm abnormalities (*41,42*), such appears not to be the case with DEHP.

The genotoxicity that is observed with phthalates by others (*3,5–7,12,13,15*) occurs at very high concentrations. Accordingly, the influences of limited solubility, impurities, or an indirect genotoxic effect resulting from primary toxicity should be considered seriously as a causative factor in these cases. For instance, in Seed's study (*6*) of phthalates using bacterial strains of *Salmonella typhimurium*, no absolute increases in the mutant frequencies in *S. typhimurium* treated with a number of phthalates were observed; increased mutagenicity was obtained only when data were corrected for viability.

On the other hand, no genotoxicity was observed in the present experiments, even at very high, toxic levels of DEHP. This suggests that toxic effects are not the only determinants influencing the genotoxic effects of phthalates in other studies. The contradictory results reported for some assay systems (*1,3–6,13–15*) suggest that variability in general experimental conditions, culture media, cell types or species could account for some of the reported ambiguities.

Technical assistance by L. Miller, J. Bayley, and V. Liu-Lee and animal care by A. Sadler, L. Saumoure, C. Picard, and C. Hunter of the Animal Resources Division are greatly appreciated. We also thank K. Nesbitt for expert typing, and Drs. E. R. Nestmann and A. F. Hanham for reviewing the manuscript.

REFERENCES

1. Simmon, V. F., Kauhanen, K., and Tardiff, R. G. Mutagenic activity of chemicals identified in drinking water. In: Progress in Genetic Toxicology (D. Scott, B. A. Bridges, and F. H. Sobels, Eds.), Elsevier/North Holland, Amsterdam 1977, pp. 249–258.
2. Ruddick, J. A., Villenueve, D. C., Chu, I., Nestmann, E., and Miles, D. An assessment of the teratogenicity in the rat and mutagenicity in Salmonella of mono-2-ethylhexyl phthalate. Bull. Environ. Contam. Toxicol. 27: 181–186 (1981).
3. Kozumbo, W. J., Kroll, R., and Rubin, R. J. Assessment of the mutagenicity of phthalate esters. Environ. Health Perspect. 45: 103–109 (1982).
4. Zeiger, E., Haworth, S., Speck, W., and Mortelmans, K. Phthalate ester testing in the National Toxicology Program's Environmental Mutagenesis Test Development Program. Environ. Health Perspect. 45: 99–101 (1982).
5. Tomita, I., Nakamura, Y., Aoki, N., and Inui, N. Mutagenic/carcinogenic potential of DEHP and MEHP. Environ. Health Perspect. 45: 119–125 (1982).
6. Seed, J. L. Mutagenic activity of phthalate esters in bacterial liquid suspension assays. Environ. Health Perspect. 45: 111–114 (1982).
7. Phillips, B. J., James, T. E. B., and Gangolli, S. D. Genotoxicity studies of di(2-ethylhexyl) phthalate and its metabolites in CHO cells. Mutat. Res. 102: 297–304 (1982).
8. Kirby, P. E., Pizzarello, R. F., Lawlor, T. E., Haworth, S. R., and Hodgson, J. R. Evaluation of di-(2-ethylhexyl) phthalate and its major metabolites in the Ames test and L5178Y mouse lymphoma mutagenicity assay. Environ. Mutag. 5: 657–663 (1983).
9. Abe, S., and Sasaki, M. Chromosome aberrations and sister chromatid exchanges in Chinese hamster cells exposed to various chemicals. J. Natl. Cancer Inst. 58: 1635–1641 (1977).
10. Tsuchiya, K., and Hattori, K. Chromosomal study on human leukocytes cultures treated with phthalate acid ester. Repts. Hokkaido Inst. Public Health 26: 114 (1976).
11. Stenchever, M. A., Allen, M. A., Jerominski, L., and Petersen, R. V. Effects of bis(2-ethylhexyl) phthalate on chromosomes of human leukocytes and human fetal lung cells. J. Pharm. Sci. 65: 1648–1651 (1976).
12. Yurchenko, V. V. A cytogenetic study of the mutagenic properties of the repellants dimethylphthalate and phenoxyacetic acid *N,N*-diethylamide. Farmikol. Toksikol. 40: 454–457 (1977).
13. Singh, A. R., Lawrence, W. H., and Autian, J. Mutagenic and antifertility sensitivities of mice to di-2-ethylhexyl phthalate (DEHP) and dimethoxyethyl phthalate (DMEP). Toxicol. Appl. Pharmacol. 29: 35–46 (1974).
14. Hamano, Y., Inoue, K., Oda, Y., Yamamoto, H., and Kunita, N. Studies on toxicity of phthalic acid esters. 2. Dominant lethal test of DEHP and MEHP in mice. Osaka-Fu Koshu Eisei Kenkyu Hokoku Shokuhin Eisei Hen 10: 1–4 (1979).
15. Autian, J. Antifertility effects and dominant lethal assays for mutagenic effects of DEHP. Environ. Health Perspect. 45: 115–118 (1982).
16. Teranishi, H., and Kasuya, M. The effects of phthalate esters on fibroblasts in primary culture. Toxicol. Letters 6: 11–15 (1980).
17. Dillingham, E. O., and Autian, J. Teratogenicity, mutagenicity and cellular toxicity of phthalate esters. Environ. Health Perspect. 3: 81–89 (1973).
18. Gesler, R. M. Toxicology of di-2-ethylhexyl phthalate and other phthalic acid ester plasticizers. Environ. Health Perspect. 3: 73–79 (1973).
19. Lee, H., and Kalmus, G. W. Cytotoxic effect of di(2-ethylhexyl) phthalate on cultured chick embryo cells. Experientia 30: 800–801 (1974).
20. Sanders, H. O., Mayer, F. L., Jr., and Walsh, D. F. Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates. Environ. Res. 6: 84–90 (1973).
21. Shiota, K., Chou, M. J., and Nishimura, H. Embryotoxic effects of di-2-ethylhexyl phthalate (DEHP) in di-*n*-butyl phthalate (DBP) in mice. Environ. Res. 22: 245–253 (1980).
22. Yagi, Y., Nakamura, Y., Tomita, I., Tsuchikawa, K., and Shimoi, N. Teratogenic potential of di- and mono-(2-ethylhexyl) phthalate in mice. J. Environ. Pathol. Toxicol. 4: 533–544 (1980).
23. Singh, A. R., Lawrence, W. H., and Autian, J. Teratogenicity of phthalate esters in rats. J. Pharm. Sci. 61: 51–55 (1972).
24. Haberman, S., Guess, W. L., Rowan, D. F., Bowman, R. O., and Bower, R. K. Effects of plastics and their additives on human serum proteins, antibodies and developing chick embryos. SPE J. 24: 62–69 (1968).
25. NTP. National Toxicology Program Carcinogenesis Bioassay of Di(2-ethylhexyl)phthalate (CAS No. 117-81-7) in F344 Rats and B6C3F1 Mice (feed study). DHHS Publication No. (NIH) 82-1773, Carcinogenesis Testing Program, NTP, NIH, Bethesda, MD (1982).
26. NCI. Bioassy of Dimethylterephthalate for Possible Carcinogenicity (CAS No. 120-61-6). DHHS Publication No. (NIH) 79-1376, Bethesda, MD (1979).
27. NTP. Carcinogenesis Bioassay of Diallylphthalate (CAS No. 131-17-

9) in B6C3F1 Mice (gavage study). DHHS Publication No. (NIH) 83-1798, Bethesda, MD (1983).
28. NTP. Carcinogenesis Bioassay of Di(2-ethylhexyl)adipate (CAS No. 103-23-1) F344 Rats and B6C3F1 Mice (feed study). DHHS Publication No.(NIH) 81-1768, Bethesda, MD (1982).
29. NCI. Bioassay of Phthalamide for Possible Carcinogenicity (CAS No. 88-96-0). DHHS Publication No. (NIH) 79-1717, Bethesda, MD (1979).
30. NCI. Bioassay of Phthalic Anhydride for Possible Carcinogenicity (CAS No. 85-44-9). DHHS Publication No. (NIH) 79-1715, Bethesda, MD (1979).
31. Gangolli, S. D. Testicular effects of phthalate esters. Environ. Health Perspect. 45: 77–84 (1982).
32. Curto, K. A., and Thomas, J. A. Comparative effects of diethylhexyl phthalate or monoethylhexyl phthalate on male mouse and rat reproductive organs. Toxicol. Appl. Pharmacol. 62: 121–125 (1982).
33. Oishi, S. Testicular atrophy of rats induced by di-2-ethylhexyl phthalate: effects of vitamin A and zinc concentrations in the testis, liver and serum. Toxicol. Letters 20: 75–78 (1984).
34. Gray, T. J. B., and Butterworth, K. R. Testicular atrophy produced by phthalate esters. Arch. Toxicol. (Suppl. 4) 00: 452–455 (1980).
35. Seth, P. K., Sriwastava, S. P., Agarwal, D. K., and Chandra, S. V. Effect of di-2-ethylhexyl phthalate (DEHP) on rat gonads. Environ. Res. 12: 131–138 (1976).
36. Oishi, S., and Hiraga, K. Testicular atrophy induced by phthalic acid esters: effect on testosterone and zinc concentrations. Toxicol. Appl. Pharmacol. 53: 35–41 (1980).
37. Foster, P. M. D., Thomas, L. V., Cook, M. W., and Gangolli, S. D. Study of the testicular effects and changes in zinc excretion produced by some *n*-alkyl phthalates in the rat. Toxicol. Appl. Pharmacol. 54: 392–398 (1980).
38. Creasy, D. M., Foster, J. R., and Foster, P. M. D. The morphological development of di-*n*-pentyl phthalate induced testicular atrophy in the rat. J. Pathol. 139: 309–321 (1983).
39. Wyrobek, A. J., and Bruce, W. R. The induction of sperm shape abnormalities in mice and humans. In: Chemical Mutagens: Principles and Methods for their Detection. Vol. 5 (A. Hollaender and F. J. de Serres, Eds.), Plenum Press, New York, 1978, pp. 257–285.
40. Hugenholtz, A. P., and Bruce, W. R. Radiation induction of mutations affecting sperm morphology in mice. Mutat. Res. 107: 177–185 (1983).
41. Komatsu, H., Kakizoe, T., Niijima, T., Kawachi, T., and Sugimura, T. Increased sperm abnormalities due to dietary restriction. Mutat. Res. 93: 439–446 (1982).
42. Cairnie, A. B., and Leach K. E. Quantitative studies of cytological damage in mouse testis produced by exposure to heat. Can. J. Genet. Cytol. 22: 93–102 (1980).
43. Schlegel, R., and MacGregor, J. T. The persistence of micronuclei in peripheral blood erythrocytes: detection of chronic chromosome breakage in mice. Mutat. Res. 104: 367–369 (1982).
44. Sokal, R. R., and Rohlf, F. J. Biometry. The Principles and Practice of Statistics in Biological Research. W. H. Freeman, San Francisco, 1969, pp. 548–620.
45. Douglas, G. R., Grant, C. E., Bell, R. D. L., Salamone, M. F., Heddle, J. A., and Nestmann, E. R. Comparative mammalian *in vitro* and *in vivo* studies on the mutagenic activity of rhodamine WT. Mutat. Res. 118: 117–125 (1983).
46. Osterloh, J., Letz, G., Pond, S., and Becker, C. An assessment of the potential testicular toxicity of 10 pesticides using the mouse-sperm morphology assay. Mutat. Res. 116: 407–415 (1983).
47. Topham, J. C. Do induced sperm-head abnormalities in mice specifically identify mammalian mutagens rather than carcinogens? Mutat. Res. 74: 379-387 (1980).